American Journal of Hematology 60:196–199 (1999)

Study of Fibrinolytic Parameters in Different Types of Polycythemia

G. Lugassy* and I. Filin

The Institute of Hematology, Barzilai Medical Center, Ashkelon, Israel, affiliated with the Ben Gurion University of the Negev, Beer Sheva, Israel

Polycythemia vera (PV) is a myeloproliferative disorder characterized by thrombotic and, less often, bleeding complications. Many mechanisms have been advanced to explain the occurrence of these complications, none of them satisfactory. We examined a cohort of 27 patients with PV, secondary erythrocytosis, and essential thrombocythemia for coagulation and fibrinolytic parameters, including euglobulin lysis test, D-dimer, and α2 antiplasmin. Ten of the 27 patients developed one or more thrombotic complications during the study. We found no clinical correlation between the studied parameters and the complications. Three patients, one of each group, with elevated serum α2 antiplasmin levels, developed severe arterial or venous thromboses. Am. J. Hematol. 60:196–199, 1999. © 1999 Wiley-Liss, Inc.

Key words: polycythemia; fibrinolysis

INTRODUCTION

Thrombosis and bleeding are frequent complications of polycythemia vera (PV). Several mechanisms have been postulated to explain these manifestations: whole blood viscosity [1], absolute platelet count [2], platelet aggregation abnormalities [3], and decreased levels of factor XII [4].

The fibrinolytic system has been given little attention in PV. Investigators have found decreased levels of prekallikrein and kallikrein inhibitors [4] and reduced levels of tissue plasminogen inhibitor antigen [5].

We conducted a prospective comparative study of some components of the fibrinolytic system in patients with PV, essential thrombocythemia (ET), and secondary erythrocytosis (SE), and looked for a possible relationship between the studied parameters and the development of thrombohemorrhagic complications among these patients.

PATIENTS AND METHODS

Diagnoses of PV and ET were established according to the polycythemia vera study group criteria [6,7]: elevated erythrocyte mass, absence of iron deficiency, no Philadelphia chromosome, no bone marrow fibrosis, and no known cause for erythrocytosis or reactive thrombocytosis. Patients with PV, ET, or SE were either treated with phlebotomy or chemotherapy, or were untreated during the 12-month follow-up. None of the patients received antiplatelet or anticoagulant therapy at the time of testing. Blood counts were performed on fresh EDTA mixed blood with an STKS cell counter (Coulter Corporation, Hialeah, FL).

For coagulation studies, blood was mixed with one-tenth volume of 3.8% sodium citrate and plasma was separated after centrifugation at 300 rpm for 20 min at 4°C. All plasma samples were stored at −70°C until used. Prothrombin time (PT), partial thromboplastin time (PTT) and fibrinogen levels were determined by an ACL 1000 coagulometer (Instrumentation Laboratory, Barcelona, Spain). For study of fibrinolysis, blood was drawn early in the morning because of the circadian variations of the fibrinolysis parameters. A D-dimer test was performed by the Latex method (Baxter Diagnostic, Dudingen, Switzerland).

*Correspondence to: Dr. Gilles Lugassy, Institute of Hematology, Barzilai Medical Center, Ashkelon, Israel.

Received for publication 5 March 1998; Accepted 4 November 1998

Euglobulin Lysis Test (ELT)

Preparation of euglobulin was as follows: the plasma euglobulin fraction was prepared by 20 times dilution of citrated plasma (0.5 ml) and acidification at pH 5.2. After leaving for 1 hr at 4°C followed by centrifugation (1,800xg) for 10 min at 4°C, the precipitate was dissolved by 0.5 ml of 0.1 M Tris Hcl, pH 7.4. All the samples were used for ELT immediately after preparation.

For the ELT, 50 μl of human thrombin (100 IU/ml) was placed in individual wells of a 96-well microtiter plate and the clot formation was initiated by adding 150 μl of the plasma euglobulin fraction. The turbidity of the wells was measured as a function of the absorbance at 340 nm every 30 min using an automatic microtiter plate reader (Sigma Seiki Inc., Japan). The assay was performed at 37°C. The absorbance data were plotted against time. All the samples were assayed in duplicate.

The α2 antiplasmin level was defined by the chromogenic method. Plasma was mixed with trisodium nitrate, 3.8% in a ratio of 9 to 1, centrifuged at 1,000xg for 10 min, and stored at −20°C until processed. Samples were mixed with a diluted buffer and incubated with 25-nkat plasmin diluted in 2.5 ml of plasmin dilutant. Detection of the residual plasmin activity on the α2 antiplasmin substrate was performed by evaluating the paranitroaniline release, monitored at 405 nm on an ACL200 coagulometer (Instrumentation Laboratory, Barcelona, Spain). The α2 antiplasmin levels were given in percentage of paranitroaniline release. Results were compared with sample values obtained from 12 healthy controls. Measurement of plasma homocysteine was performed in selected cases, using a fluorescence polarization immunassay (Abbott IMX Analyser, Abbott Park, IL). The authorization of the local and national Helsinki committees was obtained. All patients gave their informed consent prior to their inclusion in the study.

RESULTS

Twenty-seven PV, SE, and ET patients were included in the study (Table I). Thirteen patients were diagnosed with PV, 5 females and 8 males, with a mean age of 65 years (range 47–>86 years). Eight patients were diagnosed with SE, all men, with a mean age of 59 years (range 40–70 years). The erythrocytosis was attributed to smoking in all patients, after other common etiologies were excluded. Six patients were diagnosed with ET, 3 females and 3 males, with a mean age of 60 years (range 36–77 years). Twelve healthy controls, 6 females and 6 males, with a mean age of 50 years (range 38–70 years) were also included in the study. Thrombotic complications occurred in 10 of the 27 patients. Six patients had thrombosis before the study was initiated, and 4 developed venous or arterial thrombosis during the study. None suffered from bleeding. Seven of the 10 patients with thrombotic complications were in the PV group, 2 had SE, and 1 had ET. None of the controls had thrombotic or bleeding manifestations. Hemoglobin levels and erythrocyte numbers were higher in the SE group (17.4 g/dl, $5.8 \times 10^6/mm^3$, respectively) than in the PV group, treated and untreated alike (16 g/dl, $5.4 \times 10^6/mm^3$, respectively), or the ET group (13 g/dl, $4.1 \times 10^6/mm^3$). Mean hemoglobin levels in the control group were 12.7 g/dl. Platelet levels were higher in the ET group (mean $680 \times 10^3/mm^3$, range: 429–>$864 \times 10^3/mm^3$) than in the PV group (mean $270 \times 10^3/mm^3$, range: 137–$410 \times 10^3/mm^3$) or the SE group (mean $210 \times 10^3/mm^3$, range: 167–$284 \times 10^3/mm^3$). In the control group, the mean platelet count was $210 \times 10^3/mm^3$, range: 186–$336 \times 10^3/mm^3$White blood cell counts were not significantly different in all four groups. PT levels were within normal limits (above 50%) in all but 1 patient, who belonged to the chemotherapy-treated PV group. PTT levels were normal (below 38′) in all but 5 PV patients, 1 SE patient, 1 ET patient, and 1 control. Circulating anticoagulant was not found in these patients. Values for euglobulin lysis time were comparable in PV, SE, and ET patients and in the control group: 90′ in the patients' group vs. 100′ in the control group. D-dimer levels were identical within the 3 patient groups and in the control group, <0.25.

Serum α2 antiplasmin levels of the 27 patients are shown in Table I. Mean values for PV, SE, and ET patients were similar to the values measured in the control group: 104%, 107%, 130%, and 115%, respectively. Serum levels of α2 antiplasmin were elevated (above 2 sd of the normal value) in 3 patients who had suffered severe episodes of thrombosis several months prior to testing:

1. A 63-year-old female from the hydroxyurea-treated PV group had an α2 antiplasmin level of 167%. She developed deep vein thrombosis anderebrovscular accident during the study period. The hemoglobin, hematocrit, and platelet counts were within normal limits at the time of the thrombotic complications.
2. A 70-year-old male with untreated SE and a serum α2 antiplasmin level of 144%, suffered a thrombosis of the femoral artery. His hematological profile was otherwise normal.
3. A 62-year-old male with untreated ET and a serum α2 antiplasmin level of 174% developed a thrombosis in a digital artery. All other hematological parameters were normal.

Plasma homocysteine levels were normal in the two latter patients.

TABLE I. α2 Antiplasmin Levels in 27 Patients With PV, SE, ET*

Diagnosis	Treatment	Thrombotic event	Ht %	Platelets $\times 10^3/mm^3$	α2 antiplasmin %
PV	Untreated	CVA, MI	57.4	204	99
PV	Untreated	—	37.3	399	107
PV	Untreated	Recurrent CVA	34.6	328	103
PV spent	Untreated	—	25.2	137	55
PV	Phlebotomy	—	48.8	237	131
PV	Phlebotomy	MI	48	330	75
PV	Phlebotomy	—	50.4	160	98
PV	Phlebotomy	DVT	46.7	410	102
PV	Hydroxyurea	—	48	494	124
PV	Hydroxyurea	DVT	47.9	260	86
PV	Hydroxyurea	DVT, CVA	34.9	400	167
PV	Hydroxyurea	Recurrent CVA	42.5	199	102
PV	Hydroxyurea	DVT	41.6	227	107
SE	Untreated	—	51	284	89
SE	Untreated	—	51.8	250	108
SE	Untreated	Arterial thrombosis	47.4	272	144
SE	Phlebotomy	—	52.6	167	128
SE	Phlebotomy	CVA	51.9	200	100
SE	Phlebotomy	—	51.3	256	94
SE	Phlebotomy	—	51.6	228	94
SE	Phlebotomy	—	52.9	190	121
ET	Untreated	—	45.7	864	129
ET	Untreated	Arterial thrombosis	35.7	792	174
ET	Hydroxyurea	—	36.2	696	131
ET	Hydroxyurea	—	38	514	120
ET	Hydroxyurea	—	32.7	429	98
ET	Hydroxyurea	—	44.4	504	129

*PV, polycythemia vera; SE, secondary erthrocytosis; ET, essential thrombocythemia; CVA, cerebrovascular accident; MI, myocardial infarction; DVT, deep vein thrombosis; Ht, hematocrit.

DISCUSSION

PV is a clonal disorder involving multipotent hematopoietic stem cells [8]. Most individuals with PV are prone to thrombosis while few suffer from both thrombosis and bleeding during the course of the disease [9]. Furthermore, a PV patient may shift from being primarily a bleeder to being thrombosis prone as the disease progresses.

Hyperviscosity due to elevated hematocrit, thrombocytosis, abnormal platelet aggregation, and activation of coagulation cascade have all been documented in PV [1–4]. No correlation has been established between these findings and the complications encountered in PV.

Among other possible mechanisms, the fibrinolytic system has been given little attention in PV. Bick [10], in 1974, found subnormal fibrinolytic potential in 2 out of 4 PV patients. Takahashi et al. [11] found a decreased α2 plasmin inhibitor in 9 PV patients. Recently, Wieczorek et al. [12] measured reduced plasminogen activator inhibitor (PAI) antigen levels in PV patients who experienced a thrombotic event compared with normal PAI antigen levels in asymptomatic PV patients. Cohen et al. [5] confirmed the decreased PAI values in a cohort of 86 PV patients, but found no correlation with the risk of thromboembolic complications.

We examined a cohort of 27 patients with PV, SE, and ET and compared their complete blood counts, D-dimer levels, euglobulin lysis tests, and serum α2 antiplasmin values to a population of 12 healthy controls. We looked for a possible correlation between several parameters of fibrinolysis and the development of thrombohemorrhagic complications in this group of patients. Ten of the 27 patients, mostly PV patients, developed at least one thrombotic episode before or during the study.

We found no abnormalities among the tested parameters that could differentiate between asymptomatic and symptomatic patients. Mainly, PV patients who developed thrombosis had the same hematologic, coagulation, and fibrinolytic parameters than uncomplicated PV, ET, or SE patients or controls alike.

Because none of the patients suffered from hemorrhage, the D-dimer test had little relevance in our study, since this is a more reliable test of hyperfibrinolysis than of decreased fibrinolysis.

Elevated plasma levels of α2 antiplasmin were found in three patients (1 PV, 1 SE, 1 ET). These patients had developed severe venous and arterial thromboses prior to the study period. They had no evidence of chronic thrombosis that could explain an increase of α2 antiplasmin inhibitor complexes at the time of the study.

We suggest that elevated levels of α2 antiplasmin could be a risk factor for thrombosis. This hypothesis needs to be confirmed in further clinical studies.

REFERENCES

1. Thomas DJ, Duboulay GH, Marshall J, Pearson TC, Ross Russell RW, Symon L, Wetherley-Mein G, Zilkha E. Effect of haematocrit on cerebral blood-flow in man. Lancet 1977;2:941–943.
2. Dawson AA, Ogston D. The influence of the platelet count on the incidence of thrombotic and haemorrhagic complications in polycythaemia vera. Postgrad Med J 1970;46:76–78.
3. Schafer AI. Bleeding and thrombosis in the myeloproliferative disorders. Blood 1984;64:1–12.
4. Carvallo A, Ellman L. Activation of the Coagulation System in Polycythemia Vera. Blood 47:669-678, 1976.
5. Cohen AM, Gelvan A, Kadouri A, Creter D, Djaldetti M. Tissue plasminogen activation levels in different types of polycythemia. Eur J Haematol 1990;45:48–51.
6. Berlin NI. The diagnosis and classification of the polycythemias. Semin Hematol 1975;12:339–351.
7. Dudley JM, Messinezy M, Eridani S, Holland LJ, Lawrie A, Nunan T, Sawer B, Savidge GF, Pearson TC. Primary thrombocythaemia: diagnostic criteria and a simple scoring system for positive diagnosis. Br J Haematol 1989;71:331–335.
8. Adamson JW, Fialkow PJ, Murphy S, Prchal JF, Steinmann L. Polycythemia vera: stem cell and probable clonal origin of the disease. N Engl J Med 1976;295:913–916.
9. Bilgrami S, Greenberg BR. Polycythemia rubra vera. Semin Oncol 1995;22:307–326.
10. Bick RL. Fibrinolytic potential in polycythemia rubra vera. Beitr Path Bd 1974;151:401–404.
11. Takahashi H, Hattori A, Shibata A. Profile of blood coagulation and fibrinolysis in chronic myeloproliferative disorders. J Exp Med 1982; 138:71–80.
12. Wieczorek I, MacGregor IR, Prescott RJ, Ludlam CA. The fibrinolytic system and proteins C and S in treated polycythemia rubra vera. Blood Coagul Fibrinolysis 1992;3:823–826.